

ABBOTT (A.C.)

*Sewer Gas:
Chemical, Physical
and
Bacteriological Studies.*

By A. C. ABBOTT, M.D.

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With the author's compliments.

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SPECIAL REFERENCE TO THEIR APPLICA-
TION TO THE AIR OF SEWERS.

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Mr. President, gentlemen of the Congress: the subject set for discussion this afternoon, on which I have the honor to speak, is one that has attracted attention from the medical and lay world for a number of years, and opinions upon it are still at variance. When I accepted the invitation of the President of the American Climatological Association to present a paper at this meeting based upon experimental studies relating to the sewer gas question, I little realized the manifold ramifications of the task I had consented to undertake, and if the results of my efforts to obtain more light upon this important subject are not conclusive, and appear rather as indications than actual demonstrations, I look to your indulgence, and trust you may realize that a part, at least, of the shortcoming is attributable to the nature of the problem that has been attacked.

I shall not trespass upon your time with a recital of the opinions that prevail with regard to the influence of the air of sewers, drains, and cesspools in the production of disease; that you will hear from the speaker who is to follow me in the thorough way so characteristic of his writing, but shall ask your attention to a consideration of the chemistry, bacteriology and physics of the air of these localities.

A review of the literature can but leave the impression that many of the opinions advanced from time to time upon these aspects of the subject are manifestly the outgrowth of intuition, or are ideas not based upon exact methods of investigation. From such sources we might be led to believe that the air of sewers is a mysterious mixture of poisonous, gaseous products of decomposition, holding in suspension innumerable bacteria capable of producing all manner of disease, and existing under a tension that enables it to press outward in all directions, and especially into houses that are in communication with

the sewer. On the other hand, opinions that are based upon the study of this problem by precise, scientific methods demonstrate the inaccuracy of such views at almost every point that the subject has been approached. We are now tolerably well acquainted with the nature of the air of sewers, thanks to the efforts of a few who have endeavored to learn the truth on the subject, and we know that as ordinarily found it does not differ very conspicuously from the air we are accustomed to breathe. As a result of the numerous and careful studies upon the chemistry of such air *in situ* by deClambray,¹ Lethby,² Nichols,³ Miller,⁴ Russell,⁵ Beetz⁶ and others, we learn that it is ordinary atmosphere with which are mixed certain gaseous products of decomposition; and that the most conspicuous chemical difference between it and pure air is that it is usually a little poorer in oxygen, and richer in carbonic acid, ammonia and sulphuretted hydrogen than is outside air. It was demonstrated by the investigations of these observers that the proportion of oxygen in the air of all sewers examined by them was never found to be lower than 17.4 per cent. by volume, with one exception, and this occurred in a choked arm of the notorious sewer Amelot in Paris where the reduction of this gas reached to 13.8 per cent. by volume. The same observers found that the proportion of carbonic acid in the air of these sewers fluctuated between 0.12 per cent. and 3.4 per cent. by volume, and that frequently the other gaseous products of decomposition were either not to be detected with accuracy, or they were present in very small amounts.

The studies that have been made by Miquel,⁷ Petri,⁸ and Carnelley and Haldane⁹ upon the air of sewers with reference to its bacteriology are concordant in demonstrating its comparative poverty in bacteria, their number becoming worthy of consideration only under such conditions as splashing or active bubbling of the sewage, and even then rarely or never reaching the figures obtained from analyses of the air of the overlying streets or of inhabited houses.

A number of efforts have been made to determine if there exist in sewer air volatile organic matters that could be held accountable for the diseases believed by some to be contracted through the respiration of such air, and while different observers report the finding of such organic matters, the amounts in which they were detected are so small that they can readily be dismissed from consideration.

We have been led to believe, through some of the teaching on this subject, that the air of sewers exists under an active pressure, and tends to force its way into houses in connection with the sewer. Those who have studied the conditions as they are found in sewers have thus far failed to obtain any evidence whatever in corroboration of such an opinion.

Professor Chandler¹⁰ in his lectures before the Trades School Plumbing class expressed himself on this point as follows: "The common idea that gas in our sewers exerts a pressure to get out is a fallacy; I have yet to find a case to prove it." He continues—"I some time ago had a lot of pressure gauges made which were distributed among the students with the instruction to apply them wherever they could, but there has as yet been no case of pressure reported." He had a vent taken from the street sewer to his laboratory, thinking that then he could get sewer gas whenever he wanted it by simply turning on the stop cock, but no pressure has yet been indicated. He concludes: "The sewers are not tight enough to stand any pressure from gases. They are not even impervious to water."

The well known observations of Rozahegyi¹¹ upon the sewers of Munich demonstrated that in 172 observations there was on 57 occasions no movement whatever of the air in the sewers; that for 45 times when he found the current of air flowing toward the houses it was in the reverse direction 70 times; and the studies of Santo Crimp¹² upon the movements of the air of the sewers at Wimbledon demonstrated the existence of currents away from the houses on 273 days against 97 days in which it was toward them, and 88 days in which there was not sufficient movement in either direction to be detected by an anæmometer.

These studies upon the chemical, bacteriological and physical side of the subject together with many observations upon the health of those constantly exposed to the emanations from sewage, and other matters in which decomposition is in progress, are certainly not of a nature to warrant the opinion that sewer air is the dangerous factor that some believe it to be.

Among the results obtained by those who have studied the conditions found in sewers by exact analytical methods there does not appear a single conclusive demonstration that the air of sewers stands in causal relation to the diseases for which it has been held accountable.

Are we justified in considering these observations as conclusive? Do the observations made in sewers, as we find them, indicate all that is possible to occur in these places?

Is it possible to decide if the unimportant nature of this air *in situ*, as determined by chemical analysis, results from inactivity of the decomposition in progress in the sewage itself, or, is due to constant and rapid dilution of the products of active decomposition by air from without?

Can we, through laboratory methods, offer any arguments that may be of service in formulating a reasonable view upon the bacteriological

condition of this air, and its probable rôle in the spread and dissemination of infectious diseases?

Is it possible to demonstrate experimentally on animals that the gases arising from sewage or from other decomposing substances have any direct effect upon the health of the animal, or its ability to resist infection?

These represent some of the problems that we have endeavored to solve by experiments in the laboratory, problems that can only be satisfactorily answered when practically all surrounding conditions are under the control of the experimenter.

No argument is required in support of the statement, that when one enters a sewer or smells of sewage the impression made upon the senses is very different from that obtained from such actively decomposing substances as an infusion of meat, or other bodies rich in proteids, and if sewage is confined in a closed place there is often no evidence to the senses of active decomposition, and one cannot avoid the conclusion that the changes going on in sewage must frequently be relatively inactive, or else, different from those that accompany ordinary putrefaction. I have endeavored to satisfy myself as to the accuracy or falsity of this opinion by parallel experiments made with sewage, and with solutions known to undergo active decomposition. The results of these experiments, will, I think, suffice to demonstrate the relative inactivity of the decomposition frequently in progress in sewage as compared with matters more favorable to the process. They also indicate by their variability the impropriety of preconceived general notions on the subject. We find that with certain samples decomposition is so feeble that after as long as 14 days there will still remain as much as 17 per cent. by volume of available oxygen in the air of the flask and the amount of carbonic acid will have increased to only 1.42 per cent., whereas with other samples in which the sewage is mixed with slime scraped from the sides of the sewer, the gaseous exchange is much more conspicuous, in one case being quite as pronounced as that seen in meat infusions undergoing putrefaction under similar conditions (see Table I). These differences are hardly to be explained through differences in the character of the microorganisms, to the activity of which decomposition is due, for, if we remember the studies of Roscoe and Lunt¹³ upon the bacteria of sewage, they demonstrate that the majority of the different species isolated by them possess the property, when placed under conditions favorable to growth, of conspicuously reducing the amount of oxygen in the overlying air, and increasing coincidently the proportion of carbonic acid. The studies of Hesse¹⁴ have shown that this is a prop-

erty not peculiar to putrefactive bacteria, but is one that is possessed by a number of other forms not normally concerned in putrefaction. Evidently then the limited gaseous exchange that we observe, at least in some samples of sewage, is owing to unfavorable conditions offered by the sewage itself.

From another series of experiments additional evidence of the inactivity of the decomposition in sewage has been obtained. If one connects a mercurial monometer with a flask containing an actively decomposing body, such as an infusion of meat, one observes after a very few hours that a change in the level of the mercury has taken place. There is usually at first a slight depression of the mercury, due doubtless to the primary extraction of oxygen. This is quickly followed by an increase of tension, which, after a few days becomes conspicuous, often reaching in from 6 to 8 days a positive pressure of as much as 120 or 130 millimeters. I have tested two samples of sewage by this means; one consisted only of the fluid sewage as it flowed from the sewer, while the other was a mixture of this fluid with slime scraped from the bottom of the sewer. In neither case was there an excursion of the mercury much beyond what could be accounted for by fluctuations in the temperature of the surrounding air. The results of these observations as compared with a similar experiment with putrifying meat infusion are represented graphically in chart I, and, as will be seen, give testimony in favor of the opinion of Professor Chandler already quoted. In the light of experiments of this kind, conducted under circumstances most favorable to the activities of the factors concerned in causing an increase of gas tension, it is difficult to maintain that the air of so pervious a vessel as a sewer could acquire, as a result of decomposition in the sewage, sufficient pressure to enable it to force itself outward for any great distance.

During the course of this series of studies I have endeavored to detect the presence of the common products of decomposition other than carbonic acid in the air over sewage confined in sealed flasks, with irregular results. From many samples it was possible to obtain evidence of sulphuretted hydrogen, sometimes not in sufficient amount to detect other than qualitatively, while from other samples it was given off in relatively large amounts. In two samples none was detected, although the observation covered a period of six weeks in one and three months in the other case. Ammonia was practically always present, and varied conspicuously in amount. The air over several samples was tested for the presence of methyl-mercaptan, but I did not succeed in detecting it in any, though it was easily demonstrated

in the air over actively decomposing meat infusions kept under similar circumstances. We have also endeavored to obtain a closer acquaintance with the effect of ventilation upon decomposing bodies in much the same way that Erismann¹⁶ studied the gaseous products given off from decomposing faeces and urine. For sake of comparison these experiments were made upon decomposing meat infusion (50 grams meat to 1 litre of water) and upon sewage obtained from neighboring sewers.

The experiments were arranged as follows : Triple neck Woulff bottles of four litres capacity were one-fourth filled with meat infusion or sewage as the case might be, thus giving a ratio between air space and material of 3:1. Into one neck of the flask was fixed, by means of a rubber stopper, a glass tube of about 5 mm. caliber, this reached to within 2 cm. of the surface of the fluid ; into the middle neck was placed a thermometer and into the remaining orifice was fixed a short glass tube also of 5 mm. calibre. Both tubes were provided at their outer ends with hermetically closing stopcocks. All joints about the stoppers were sealed with paraffin. By this arrangement the air was caused to enter the flask by the longer, pass over the fluid and leave by the shorter tube. The air did not bubble through the fluid but passed over it. The entrance to the flask was guarded by an absorption flask containing pumice stone saturated with a 50 per cent. solution of potassium hydroxide, so that on entering the flask the air was free from carbonic acid. Between the exit tube of the flask and the aspirator, the absorption apparatus proper was arranged as follows : the air passed from the vessel containing the infusion or sewage first through two calcium chloride tubes, to dry it ; it then passed through two U-formed absorption tubes containing pumice stone saturated with copper sulphate and dried, according to the directions of Fresenius for estimating sulphuretted hydrogen gravimetrically (see Fresenius' Quantitative Analyse, Vol. I, p. 505, also *Zeit. für analytische Chemie*, 10-75), it next passed through (during the first three or four days) Pettenkofers' tubes containing barium hydroxide solution ; these were, however, abandoned and 2 absorption tubes containing pumice stone saturated with 50 per cent. caustic potash solution were substituted and the estimations made gravimetrically. To avoid error from loss of moisture these tubes were guarded at their exit by a layer of granular calcium chloride 4 cm. deep, separated from the pumice stone by a layer of asbestos. The air next passed to a gas meter that registered its volume in litres and thence to a Chapman water pump.

The Pettenkofer tubes and barium hydroxide solution were discarded for the reason that, where large quantities of carbonic acid are given off, it is difficult to say when the solutions are saturated, and if a second and third tube be added to indicate this point we have no trustworthy means of determining if any, or what proportion, of the precipitated barium carbonate in the first tube is redissolved by the excess of carbonic acid passing through it.

As the aspiration of air over the decomposing matters covered a number of hours between the estimations, usually about 24, and the temperature of the room was constantly changing, it was not thought advisable to reduce the air volumes to normal conditions from such data, so that in this respect there is a slight error in the results, an error that I hardly think could have been eliminated without having known the temperature of each litre, or small multiple thereof, that passed through the apparatus.

In the accompanying tables (II and III) will be seen the data obtained from continuous observations made in this way upon air passed over decomposing meat infusion (Table II) and upon air passed over sewage (Table III). In Charts II and III these results are represented graphically.

As a result we find the amount of carbonic acid given off from sewage is constantly smaller than that obtained from the meat infusion, while at times the proportion of sulphuretted hydrogen from the samples examined became suddenly comparatively high. Whether the large amount of sulphuretted hydrogen given off by the sewage is due to the presence in the samples of sulphur compounds more easily converted into sulphuretted hydrogen than those in the meat infusion; whether in the case of the meat infusion it was held back through combinations that it may have formed with other non-volatile or less volatile substances, or whether the difference is the result of peculiar fermentative processes in the one case that were not in operation in the other, as the observations of Petri and Maasen¹⁶ and of Stagnitta-Balisteri¹⁷ have shown to be possible, I cannot say, the difference is, however, striking, as reference to the charts will show. With the sewage, as with the meat infusion, a sudden but temporary fall of temperature was almost invariably followed by a conspicuous increase in the amount of sulphuretted hydrogen in the air, while the carbonic acid remained unaffected (see tables and charts). This sudden diffusion of sulphuretted hydrogen from the fluid, in which it, in part, seemed to be loosely dissolved, into the overlying air is doubtless to be explained through the difference of specific heat of the two bodies that are in juxtaposition, the sudden fall in temperature favoring diffusion from the

fluid of high, to the air of lower specific heat. Another point of interest that is brought out in these experiments is that gentle agitation of the fluids likewise increased the amount of sulphuretted hydrogen in the air, but had little effect on the carbonic acid. This is particularly the case with the sewage, where there was always a tendency to the formation of very dense pellicles on the surface, which seemed to prevent, when intact, the escape of this gas from the fluid. The breaking of such pellicles was almost invariably followed by an increase of sulphuretted hydrogen in the overlying air (see Chart III). When the solutions were kept perfectly still, and there was but little fluctuation of temperature, steady aspiration of the air over them showed a pretty constant production of carbonic acid, while the amount of sulphuretted hydrogen would often be reduced to nothing. A summary of these observations shows us that air passed over such an actively decomposing body as an infusion of meat, when ventilation of the chamber containing it is conducted at such velocities that the entire air is changed from 20 to 60 times in 24 hours, carried off as a mean of 23 observations on as many days, 29.96 parts per 10,000, by volume, of carbonic acid, the highest relative amount on any single day being 75.97 parts per 10,000 with the ventilation equivalent to renewal of the air 24 times in 24 hours, and the lowest being 15.1 parts per 10,000 with ventilation at the rate of 19 renewals of the air in 24 hours. When ventilation was reduced to practically nothing, that is, when the total volume of air was renewed only from one to three times in 24 hours, the mean amount of carbonic acid reached the enormous proportions of 682.7 parts per 10,000. Air similarly aspirated over sewage at velocities that insured complete renewal of the contents of the chamber from 12.5 to 80 times in 24 hours gave as the means of all analyses in each group of experiments the following relative proportions :

Group 1—From December 11th to 30th inclusive=8.61 parts per 10,000.

Group 2—From January 10th to 19th inclusive=15.4 parts per 10,000.

The highest relative amount found was at the beginning of group 2, when with a renewal of the air 28.8 times in 24 hours, 43.87 parts per 10,000 were obtained, while the lowest amount found was in group 1, when with a renewal of the air 56 times in 24 hours only 3.31 parts per 10,000 were present. Expressing in the same terms the extremes of the results of analyses made *in sewers* by the observers quoted, we find that carbonic acid was found to fluctuate between 12 and 340 parts per 10,000, while often other bodies were present in hardly

appreciable amounts. Under all conditions, therefore, not only in closed chambers, but under continuous ventilation (though varying in its rate), the decomposition in the samples of sewage examined, as evidenced by carbonic acid, its most conspicuous product, was usually of much less intensity than the decomposition of substances more favorable to the processes.

When it finally became evident that the supposed poisonous properties of the air of sewers could not be attributed to the substances that investigation had shown to be present, in the amounts that they were seen to occur, attention turned to the probable existence of a substance or substances, not included in these results, that might possess disease-inducing peculiarities. By some these bodies are assumed to be of a volatile organic nature, possibly closely related to the substituted ammonias; by others they are said to be carbo-ammoniacal in nature; by still others to be albuminoid; but in fact little definite is known as to their existence or of their nature. From time to time methods have been devised for their isolation with the result that substances were obtained that gave certain reactions common to organic matters, that is, of decolorizing potassium permanganate, or of being oxidized by it to ammonia. A detailed discussion upon the relative merits and accuracy of the various methods that have been employed would be out of place here. It will suffice to say that in the air over decomposing matters, just as in the open air, the air of rooms, and also in water, there may and do exist substances capable of reducing potassium permanganate, but which are not of necessity strictly organic, or of such a subtle nature that they cannot be easily detected by ordinary methods of analysis. The simple statement, therefore, that something has been found that decolorizes solutions of potassium permanganate, means little or nothing as to the nature of the substance. If, however, a body or group of bodies are found which, when oxidized, result in compounds that we know can come only from materials containing elements characteristic of certain groups, we then have a clue, at least, as to their general character. The oxidation, for example, of organic substances to ammonia leaves no doubt that the oxidized bodies were of nitrogenous nature. Advantage has been taken of this and methods have been devised for absorbing from the air its volatile principles and determining what proportion of them contain nitrogen, both in the form of free ammonia, and of *potential* or *albuminoid ammonia*—that is, nitrogenous matter convertible into ammonia, which latter is the body assumed to possess poisonous peculiarities. Of the various devices that have been recommended for their estimation the one suggested

by Professor Remsen¹⁸ was selected as open to least objection, in so far as the chemical processes are concerned. It consists in aspirating the air that is to be studied through glass tubes containing granular pumice stone saturated with pure distilled water; these serve as absorbers for free ammonia, ammoniacal basis, and presumably other gaseous nitrogenous matters. When the desired quantity of air has been drawn through such absorbers their contents are transferred to carefully cleansed retorts or distilling flasks containing a measured amount of pure distilled water; the free and albuminoid ammonia of this mixture is now determined by the well known method of Wanklyn, Chapman and Smith. By deducting from the totals now found the amounts of free and albuminoid ammonia previously determined for the water used, the balance represents the amounts of these bodies that were present in the volume of air aspirated through the absorber.

There are many details to be observed in the performance of this method: the flasks and condensers must be absolutely clean and an estimate must not be undertaken until it is possible to obtain from the apparatus distillates that give no reaction for ammonia when tested with Nessler's reagent in nesslerizing tubes 31.5 cm. long and 1 cm. internal diameter. (Recommended by Drown.¹⁹) The absorbing tubes 13 cm. long and about 8 to 10 mm. inside diam. must be cleansed in a hot solution of sulphuric acid and potassium bichromate, after which is it our custom to rinse in hot water and finally pass steam through them for at least two hours. The pumice stone, broken and carefully sifted until the average size of all granules is about that of a linseed or a little larger, is heated in a covered platinum dish until red hot and kept at this temperature (in our experiments), over night, at least. It is then filled into the absorbing tubes. All rubber tubing, rubber stoppers, glass connections, etc., were cleansed first in boiling water and finally by passing steam through them.

The nesslerization of all distillates and standards did not take place until they had stood, carefully protected from the air by steamed corks, for 18 to 20 hours in order that they might all be of the same temperature (see Hazen & Clark²⁰), and it is advisable, before beginning the work, to make up a sufficient quantity of Nessler's reagent to last throughout the entire series of experiments.

The distilled water used for dissolving out the materials contained in the absorbers must be carefully prepared, and in each experiment an analysis of the amount of water used in that experiment must be made, the result representing the correction (subtractive) to be made from the total results of the experiment.

For further particulars we must refer to Prof. Remsen's original paper. (See bibliography.)

Though objections have been raised to this method because of the extraordinary care necessary to avoid errors, it has, nevertheless, appealed to us as the one most likely to give concordant results when properly manipulated. Like all other methods that have been devised for this purpose, however, it affords no accurate information upon the nature of the substances retained in the absorber beyond the fact that they are or are not nitrogenous and are or are not convertible into ammonia by the processes to which they are subjected.

We have applied this method to the study of the air over decomposing meat infusions and to that over sewage, and for purposes of comparison, to outside air and air of the laboratory. The results have not however agreed with those of other observers using other methods, or with those of Professor Remsen himself using the same method. Throughout, our results have been uniformly lower than we had anticipated they would be. Of 31 estimates made upon the outside air, the air of the laboratory, air from over decomposing meat infusions and the air from over sewage, we have failed to obtain evidence of the existence of more than a trace of gaseous, nitrogenous, organic matters, other than ammonia, the amounts being constantly so small as to fall far below the permissible limits of experimental error.

Of 8 analyses of the open air obtained from just outside the second story of our laboratory not one showed the presence of nitrogenous organic matter, convertible into ammonia by the method used, though from 35 to 355 litres of air were drawn through the absorber. The amount of free ammonia was so low as to fall easily within the category of a "trace."

Of 5 determinations made upon the air of my own room in the laboratory negative results were obtained in all, though from 31 to 207 litres of air were drawn through the absorber. Free ammonia was present in barely a trace, and in one analysis none was detected.

Of 6 experiments in which air was conducted *over* sewage, confined in a closed chamber, evidence of the presence of volatile, nitrogenous, organic matters was obtained in 3, the highest amount expressed as albuminoid ammonia being 0.004 of 1 milligramme for 36.1 litres ; while in the remaining three experiments no trace of such bodies could be detected.

Of 7 experiments made by allowing air to *bubble* through sewage before passing to the absorber, the presence of volatile, nitrogenous, organic matters was demonstrated in 3, the remaining 4 being negative. The highest amount found in terms of albuminoid ammonia was 0.005 of 1 milligramme for 866 litres of air.

Of 4 experiments in which from 178 to 337 litres of air were aspirated *over* decomposing meat infusion, evidence of organic matter was obtained in all, the highest amount being 0.0172 of 1 milligramme of albuminoid ammonia for 337 litres; while in one experiment in which 143.2 litres of air were caused to bubble through decomposing meat infusion before passing to the absorber only 0.0019 of 1 milligramme of albuminoid ammonia was obtained.

These figures are low, far lower than those given for organic matter in the air by other observers, and yet I am convinced that they are still higher than they should be, the excess being due to errors during manipulation, some of which can hardly be avoided, while others seem explainable.

I account for the discrepancy between these results and those of Professor Remsen in part through the fact that in making the distillations he assumed that all free ammonia was given off in the first 100 c.c. of the distillate that passed over, and that all ammonia that now appeared, after the addition of the oxidizing agent, was derived from organic matter of an albuminoid nature.*

In my experiments with sewage and with decomposing meat infusions, I was surprised to notice that, in the distillation of the contents of the absorbers for *free* ammonia, bodies that reacted with Nessler's reagent similarly to ammonia were often coming off at the end of 100 c.c. of distillation in such quantities that the process was continued without the addition of the oxidizing agent. The result was that in these analyses, particularly, this body often persisted until as late as 350 c.c. of distillation. The addition now of the oxidizing agent (alkaline potassium permanganate) showed that whatever was left in the retort was either not convertible into ammonia, or if so, in very minute traces.

Because of the volatile nature of ammonia, we were inclined to the opinion that this body must have been something that gave with Nessler's reagent a reaction the same as that of ammonia but which was not ammonia pure and simple, differing in its physical properties at the boiling temperature. A series of control experiments, however, demonstrated that contrary to opinion, ammonia, as derived from pure ammonium chloride in the presence of sodium carbonate at the temperature of 100° C., does not all come off in the first 100 c.c. distilled over, but is being given off up to the end of 200 to 350 c.c. of distillation, according to the amount originally present. (See Table IV.) In the same series of control tests a few experiments were also made upon

* National Board of Health Bulletin. Washington, D. C., Sept. 11, 1880, Vol. 2, No. 11, page 518.

methylamine and trimethylamine, alone and mixed together, and mixed with ammonia, and as the results show, they behave in all essential respects identical with ammonia under the conditions employed.

It is manifest that unless this point be borne in mind a large proportion of the ammonia obtained after the addition of the oxidizing agent, if this be done at the end of 100 or 150 c.c. of distillation, is not of necessity ammonia derived from bodies of an albuminous nature but is ammonia, or substituted ammonias, that could have been obtained without oxidation. It seems, therefore, probable that some, at least, of the albuminoid ammonia found in Professor Remsen's work may have been free ammonia or its substitution products.

An example from one of several of my experiments may serve to make this point a little clearer and to demonstrate the possible error if what has been said is not taken into consideration. On Jan. 8th, '94, 143.2 litres of air were aspirated through a decomposing meat infusion and thence through the absorber. The evidence of ammonia in large quantities was so conspicuous that 750 c.c. of pure distilled water was mixed with the contents of the absorber, instead of 500 c.c., as was more commonly the practice. The distillation for free ammonia was made in fractions of 50 c.c. each, and up to and including the 7th fraction, that is at 350 c.c., there was still evidence of free ammonia being given off, the 7th distillate containing 0.002 mg. The 8th fraction gave no reaction for ammonia. The test for albuminoid ammonia was then made, resulting, after corrections for water used, in the total of—

$$\left. \begin{array}{l} 1.540 \text{ milligrams free ammonia} \\ 0.0017 \text{ " albuminoid ammonia} \end{array} \right\} \text{ for 143.2 litres of air tested.}$$

Had, however, the results been calculated upon the assumption that all free ammonia had been given off at the end of 100 c.c. of distillation, it is manifest that all that followed would have gone to the credit of albuminoid ammonia and the apparent amount of organic matter in the air would have been far greater than the case warrants. In short, the results calculated in this way would have indicated the presence of

$$\left. \begin{array}{l} 1.341 \text{ milligrams free ammonia} \\ 0.2007 \text{ " albuminoid ammonia} \end{array} \right\} \text{ in the 143.2}$$

liters of air analyzed, in other words, the figures indicative of the presence of organic matter as expressed by albuminoid ammonia would have been more than a hundred times too high; and when the results of all my experiments are compared with what they would have been had I calculated albuminoid ammonia from the end of

100 c.c. of distillation I find that throughout they would have been from 40 to 100 or more, times greater than they are.

In the same way I propose to explain what I mean by saying that my own results are too high. A few examples will suffice for this.

On Jan. 4, 1894, two experiments were made with air that had bubbled through sewage. In the first, 320 litres, and in the second, 866 litres were tested. I was not aware at the time of the persistency of free ammonia in the boiling solution so that the estimation for albumenoid ammonia begun at the end of 200 c.c. of distillation. On the following day the fractions were nesslerized with the following results:

<i>1st Analysis</i> —1st fraction = 0.352				} Free ammonia total = 0.4265.
2d	"	=	0.060	
3d	"	=	0.012	
4th	"	=	0.0025	
1st fraction = 0.0045				} Albumenoid ammonia = 0.0045.
2d	"	=	0.000	
3d	"	=	0.000	
<i>2d Analysis</i> —1st fraction = 0.440				} Free ammonia total = 0.590.
2d	"	=	0.120	
3d	"	=	0.024	
4th	"	=	0.006	
1st fraction = 0.0055				} Albumenoid ammonia = 0.0075.
2d	"	=	0.002	
3d	"	=	0.000	

Water used in these analyses. Subtractive correction for preceding figures.

1st fraction = 0.008				} Free ammonia, total = 0.0095.
2d	"	=	0.0015	
3d	"	=	0.000	
1st fraction = 0.0025				} Albumenoid ammonia = 0.0025.
2d	"	=	0.000	
3d	"	=	0.000	

It is, I think, probable from these figures, that a portion, at least, of the results given for albumenoid ammonia really represents free ammonia. Just how much it is impossible to say. When this became evident 284.5 litres of air in one and 387.7 litres in another experiment were aspirated through *the same sewage*. The absorbers were mixed with 750 c.c. of distilled water and *eight* fractions of 50 c.c. each were distilled off for free ammonia with the following results:

1st Experiment—1st fraction = 0.120			} Total free ammonia = 0.1755.
2d	"	= 0.036	
3d	"	= 0.014	
4th	"	= 0.004	
5th	"	= 0.0015	
6th	"	= 0.000	
7th	"	= 0.000	
8th	"	= 0.000	

2d Experiment—1st fraction = 0.180				} Total free ammonia = 0.2740.
2d	“	=	0.056	
3d	“	=	0.024	
4th	“	=	0.010	
5th	“	=	0.0025	
6th	“	=	0.0015	
7th	“	=	0.000	
8th	“	=	0.000	
1st fraction = 0.0038				} Total albumenoid ammonia = 0.0038.
2d	“	=	0.000	
3d	“	=	0.000	

The 750 cc. of water used contained—

Free ammonia	= 0.0143	} Subtractive correction for the two preceding analyses.
Albumenoid ammonia	= 0.0038	

It is not only seen that free ammonia was coming off as late as the fifth fraction in the first and the sixth fraction in the second experiment, but also that if distillation is continued until it ceases to appear before the test for *albumenoid* ammonia is begun, the figures indicative of the existence of this body become very much diminished.

A comparison of the results of these two experiments, calculated for the same volume of air in both cases will, I think, impress this point more clearly.

In the first pair of determinations there was evidence of albumenoid ammonia for 1000 cu. meters of air in the following amounts :—

1st Experiment	= 6.25	milligrams per 1000 cu. meters.
2d	"	= 5.78
	"	"
	"	"
	"	"
	"	"

While in the second pair, made with air bubbling through *the same sewage*, but with the point already made borne in mind, we obtained albumenoid ammonia in the following amounts :—

1st Experiment = 0.352 milligram per 1000 cu. meters.
 2d " = 0.000 " " 1000 "

The means of the results of each pair standing in the relation to one another as 34:1, an error that we think worthy of consideration.

Still another reason for believing that my results are too high has been brought out in several experiments in which a group of absorbers were used, *i. e.* in which the air passed through a series of two or three absorbers. In these experiments care was taken to prepare all absorbers in identically the same manner and it was not infrequent to find, though in exceedingly small amounts, that apparently more albumenoid ammonia was obtained from the second or third absorber than from the first. An example—on Jan. 12, 1894, 203.8 litres of air were drawn over a decomposing meat infusion and through two absorbers. Analysis of these absorbers gave for the *first* 0.0007 mg. and for the *second* 0.0022 mg. albumenoid ammonia, more than three times as much as was found in the first.

In consequence I cannot avoid the opinion that, though the method has been performed with the greatest care, the results obtained by me for albumenoid ammonia are more apparent than real and that the figures presented for the amounts of organic matter as indicated by the albumenoid ammonia test are in part, at least, due to unavoidable error during manipulation. The results of these analyses are given in Table V, column 7.

In addition to this method I have endeavored to employ the one suggested by Carnelley and Mackey²¹ in which the quantity of organic matter is determined colorimetrically by its decolorizing action upon solutions of potassium permanganate, but the results were so unsatisfactory that it was abandoned after a few trials. The color of the standard solutions recommended by them is so intense that errors of considerable magnitude may often exist without being recognizable; moreover, in the air in which we were interested so many reducing bodies, other than those of an albumenoid nature, were present that decolorization of the solution exposed to such air afforded evidence of but little value.

From the studies that we have made, and we consider them a fair test, it must be concluded that we have no good evidence of the existence of volatile, nitrogenous, organic matters in the air other than ammonia or its substitution products. A series of experiments are under way to determine if it is possible to differentiate between ammonia and its substituted compounds in the small amounts that they appear in the air; and while the outlook is promising I hardly think the work sufficiently far advanced to justify positive statements as

yet. It is manifest, I think, that the observation on the air of sewers *in situ* as well as those here presented, demonstrate that, as compared with other substances, the decomposition that goes on in sewage and the consequent pollution of the overlying air is much less than we are warranted in assuming. It seems also plain that we are not justified in forming anything more than a very rough conception of what is in progress, or what can occur under the conditions afforded by sewers and their contents. By the very nature of things these conditions undergo the widest range of variation. The composition of the sewage itself varies not only in different sewers, but in the same sewer at different times in the day; the dilution of the gaseous products of decomposition and fermentation, through the manifold portals of ventilation and diffusion, changes constantly with fluctuation in temperature, and particularly with the force and direction of the wind; the pollution and dilution of the air of the sewer through leakage into it of air from the surrounding soil is not the same for all geological formations through which the sewer may run, and it does not seem unlikely that some of the waste products that gain access to sewers, especially from various manufactories may play an important part in influencing the activity or character of the fermentations that are to occur. When viewed from these standpoints we must admit the impropriety of conceiving the air of sewers to be a specific compound, or one that is even likely to approach constancy in its composition.

When it long ago became evident that the potency of sewer air for the production of disease could hardly be explained by its chemical composition, attention turned to its biological characteristics, and it was assumed because sewage contained countless bacteria, and more or less frequently bacteria of a pathogenic nature, that the air over such sewage must also of necessity contain these organisms. Since we have learned that many of the diseases formerly believed to result from the respiration of the air of sewers are of a specific nature and depend for their existence upon the presence in the tissues of particular forms of micro-organisms, the opinion has been advanced that the part played by sewer air is that of a carrier of these infective particles from the sewage to the individual who has become infected. Have we any evidence in support of this opinion? None that is based upon reliable methods of investigation. Unfortunately we know little or nothing of the *nature* of the bacteria found in the air of sewers. The information that has been derived from studies made upon such air relates to the *number* of bacteria present, and this, as already stated, is demonstrated to be usually lower than that for the

open streets and inhabited houses, and we have no trustworthy evidence that pathogenic bacteria have ever been detected in sewer air. Notwithstanding this, no less of an authority on sewage than Mr. Corfield,²² as late as 1892, writes that, in addition to typhoid fever, proof has been adduced that diphtheria, erysipelas, pyæmia, septicæmia, and puerperal fever occasionally result from exposure to such air. If this be true, an agent possessed of such manifold potencies for the production of disease should certainly call for much more careful study by exact methods than it has thus far received. The teaching that, because materials containing organisms capable of producing disease are constantly gaining access to sewers, that the air of these sewers of necessity contains such organisms also, is simply an opinion; it is not supported by observations that lead us to accept it as fact. With the demonstration by Naegeli²³ that bacteria do not arise from unmolested moist surfaces, or those in which agitation is moderate, though decomposition may be in active progress, and the confirmation of this observation by Pumpelly²⁴ and many others, the opinion was suggested that perhaps bubbling and splashing that may occur in putrefying solutions might be instrumental in wafting bacteria from such solutions into the superincumbent air. This opinion was very largely formulated from the results of experiments by Professor Frankland²⁵ who caused active bubbling to occur in solutions of lithium chloride by generating carbonic acid in them and testing the superincumbent air spectroscopically for lithium. By this method he was able to detect the presence of lithium in the air at the mouth of a conducting tube 21 feet long. He found also that the lithium particles passed through a layer of coarse charcoal five inches thick, placed in the tube. From these studies he concludes "that the breaking of minute gas bubbles on the surface of a liquid . . . is a potent cause of the suspension of transportable liquid particles in the surrounding air; and, therefore, when through the stagnation of sewage or constructive defects which allow of the retention of excrementitious matters for several days in the sewer, putrefaction sets in and causes the generation of gas, suspension of zymotic matters in the air of the sewer is extremely likely to occur."

The experiments of Pumpelly demonstrated that air aspirated through decomposing fluids at so low a velocity as $\frac{2}{3}$ of a litre per day always carried bacteria with it into another flask with which it was in communication.

Mr. A. E. Truby, student of bacteriology in our laboratory, has made a series of experiments under my direction on this point, the results of which, while in the main confirmatory of the experiments

of preceding investigators, afford some additional light upon the subject. It was found, as others before had found, that with artificially produced bubbling, resulting from the aspiration of air through solutions containing bacteria, that a certain number of these are thrown into the superincumbent atmosphere, and some of them are carried in the direction of the main air current. The number of bacteria carried is dependent upon several factors, the principle being the quantity of air aspirated and the velocity at which it is drawn through the decomposing matters. He found that when from 5 to 7 litres of air were aspirated through solutions containing known species of bacteria, that none of these bacteria were transported into a flask of sterilized medium placed 1 meter distant at the end of a connecting tube of 15 millimeters inside diameter when the velocity of the air current in this tube did not exceed 8.6 cm. per minute, but that if the velocity was doubled, bacteria were carried over this distance in every case. On the other hand, in five experiments in which the length of the tube connecting the decomposing infusion and the flask of sterilized medium was increased to 4.5 meters, 7 litres of air with the velocity increased *30 fold* were aspirated through without bacteria being carried this distance in three out of five cases (for details see Table VI). Do experiments of this nature in any way simulate conditions naturally found in sewers, and do results obtained from such experiments afford a trustworthy basis for the assumption that because of splashing and bubbling in the sewer, bacteria are of a necessity thrown into overlying air and conveyed through the manifold ramifying arms of the sewer into houses in connection with it? To me it seems most unlikely. In the experiments to which reference is made there is always a single direct current of air passing through the infected solutions, causing more rapid and vigorous bubbling than I have ever been able to detect, even in the most actively decomposing infusions, and passing thence for a longer or shorter distance through straight or slightly bent smooth tubes directly into a medium that is to demonstrate the presence of bacteria that the air may contain. There is *nothing* unfavorable to the success of such a demonstration when conducted within reasonable limits.

With the hope of approaching more closely to natural conditions I arranged a number of experiments in a different way, the object being to determine if in a closed chamber from which air currents were excluded bacteria did arise from substances undergoing bubbling as a result of decomposition. To this end a large bell jar of 40 centimeters diameter, 28 centimeters high and of approximately 37 litres capacity was employed. Under it was placed an open glass dish of

about 11 centimeters in diameter and 7 centimeters high containing a fluid culture of bacteria, and arranged around this were Petri plates containing sterilized gelatine, from 4 to 7 having been used so that there was an exposure of from 310 to 550 square centimeters of nutrient gelatin upon which bacteria could fall from the air. Bubbling was brought about in two ways. First, spontaneously, by the addition to the culture of from 4 to 5 per cent. of glucose and then inoculating it with ordinary baker's yeast, and second, by the propulsion of air through it by means of a glass tube passed through an opening in the side of the bell jar. In both cases the bubbles arose to the surface of the fluid, burst and directed whatever was disengaged from the fluid straight up, the direction not being influenced by steady currents in one or another direction to any greater extent than could be avoided. Because of the manifold chances of contamination during the manipulation of so much bulky apparatus, this is hardly an ideal bacteriological experiment, but the results that have been obtained are, I think, of interest, for I believe they approach more nearly to what we may expect under conditions that are found in nature.

As a result we found that when a pure culture of an easily recognizable organism—the *bacillus prodigiosus*—was placed in a wide open vessel in the chamber and bubbling or foaming was induced through the action of yeast upon glucose, not a single colony of this organism appeared upon either of seven plates exposed in the chamber for *three* days. There did, however, appear a few colonies of other organisms, principally moulds, but similar results to this were obtained in a control experiment made in the same chamber from which the culture was absent, so that probably these colonies represented accidental contaminations.

When, on the other hand, bubbling was produced by propelling air through a similar fluid culture, at even so low a velocity as $3\frac{1}{2}$ litres in 6 hours, the bubbles rising to the surface at the rate of 22 per minute, colonies of the organism were seen on the plates in varying numbers, from 12 to 38 per plate, on *the following morning*. As with the preceding experiment, a few foreign colonies were also present.

From these observations it is clear that the number of bacteria thrown into the air as a result of bubbling from spontaneous fermentation is probably very much smaller than results obtained from artificially produced bubbling would lead us to infer. Let us now compare these observations made in closed chambers under conditions within our control with the phenomena that are presumably occurring in sewers. In the first place, as ordinarily constructed, sewers do not occasion very much splashing of their contents except, perhaps, at

isolated points. Secondly, there is no doubt that bubbling does occur, but it is probably feeble, at any rate I have never seen it to be active in sewage confined in vessels under temperature favorable to the process. Thirdly, the direction of air currents in sewers, as we know from the observations of Rosaheigyi and of Crimp (l. c.), is more often toward the mouth of the sewer than in a reverse direction, and almost as frequently there is no current at all. But let us suppose that a current did exist and always in the direction of the house, does it seem reasonable to suppose that this air, relatively poor in bacteria of all kinds, as experiment has shown it to be, would be likely to convey pathogenic bacteria, the presence of which is doubtful, from the sewer into the small branches, from these into still smaller branches, all moist, all presenting deviations from the straight line, and thence through soil pipes into houses, through water traps, or what is considered more dangerous, pin hole openings, the work of time or the careless plumber? The conditions that it would meet on its passage from the sewer into houses are not such as to favor an *increase* in the number of bacteria in it, for all experiments agree in demonstrating that such particles are not dislodged from moist surfaces even by winds of more than ordinary velocity. On the contrary, everything is favorable to a diminution in the number of organisms; with every change in the direction of the pipes conditions present that are favorable to the deposition of suspended bacteria upon the opposing moist surfaces, or their gravitation to the bottom, just as dust particles become deposited under similar circumstances. On this point the work of Carnelley and Haldane²⁶ demonstrated the interesting fact that in passing through a tube along the bottom of which water was flowing the air gradually lost a large proportion of its bacteria. As a mean of 7 experiments they found that in flowing as short a distance as 5 feet under these circumstances the number of bacteria in the air was reduced a little more than 50 per cent., and they conclude "that it seems almost unreasonable to suppose that micro-organisms can enter a house with the air of sewers, unless the draught is very strong and rapid." Carmichael²⁷ has also conclusively demonstrated that the intervention of water traps is a reliable safeguard against an invasion of bacteria, and that under ordinary circumstances they permit of less than 0.01 of a grain of gaseous matters to pass through them.

But why consume your time with arguments against the possibility of this air conveying bacteria from sewers into houses when there is nothing definite to lead us to believe that the bacteria of such air are pathogenic?

If the limited studies that have been made upon the destiny of pathogenic bacteria, when in competition with the more vigorous saprophytic forms, are capable of general application, we may reasonably assume that the conditions existing in sewers are hardly favorable to the prolongation of the life of these organisms when deposited in sewers. Surely if this is not the case, or if they are not rapidly swept away with the current of sewage, which seems most improbable, we should observe the much more frequent occurrence of acute infections among the workers in sewers than is indicated by the results of statistical studies on the subject. To meet this latter point it has been suggested that the workers in sewers acquire a tolerance to the conditions by which they are surrounded; this is plausible in so far as the gaseous matters in the air are concerned, but I fancy no one would as yet feel justified in advancing this hypothesis with regard to the organized, infectious agents, if they were as numerous, and constantly present in the air of sewers as some would have us believe.

The more conservative of those who are inclined to the belief in a causal relation between the air of sewers and pathological conditions are gradually coming to the opinion that it is not directly concerned in the production of disease, but that its continuous respiration in some way interferes with the normal, vital resistance of the tissues and thus renders them more susceptible to infections to which they may be exposed. In very recent times observations have been made that may prove to have a most important bearing upon this opinion. The studies of Sanarelli²⁸ upon experimental typhoid fever resulted in the interesting demonstration that animals not normally susceptible to this disease could be rendered susceptible to inoculation with the bacillus of typhoid fever by previous injection into their tissues of the products of growth of several organisms not normally concerned in disease production, among these being the products of ordinary putrefactive bacteria. While it is true that such procedures do not simulate very closely the respiration of sewer air or air from other decomposing matters, still, they demonstrate that in concentrated form the products of decomposition have a decidedly depressing action upon the resistance of the animal into which they are introduced.

Of still more importance in their bearing upon the subject are the observations of Alessi²⁹. He found that animals not normally open to infection by the *bacillus typhi abdominalis* became so after varying periods of exposure to the air of cesspools, and that the susceptibility was highest during the first two weeks of exposure, after which the

animals seemed to gradually gain a tolerance and were then less susceptible to infection.

I have also endeavored to get some light on the subject by experiments on animals but my work on this line was begun so late that I am hardly prepared to do more than mention it.

The experiments were arranged with the idea of determining if prolonged respiration of the gaseous products of decomposition would have any effect upon the health of animals, or their resistance to infection. With this in view 4 animals (3 guinea pigs and 1 rabbit), were confined in glass chambers through which was aspirated air that had bubbled through decomposing infusions of meat; as this was the only air obtainable by the animals for respiration, and as the infusions through which it bubbled were advanced in putrefaction, it is fair to assume that the volatile products of decomposition reached the animals in a tolerably concentrated form. Of the three guinea pigs thus confined one (Pig A), was inoculated subcutaneously with fluid cultures of the bacillus typhi abdominalis twice, and subsequently into the peritoneal cavity with a similar culture twice. The first inoculation was subcutaneous and was made after the animal had been respiring the gaseous products of decomposition for 14 days; the last was into the peritoneal cavity and was made 6 days before death. The animal lived 30 days from the beginning of the experiment, having been confined in the chamber for 16 days of this period. It diminished in weight during this time from 460 to 300 grams, and had at one period a sudden fall in temperature to 35° C., immediately following inoculation. During the balance of the time the temperature fluctuations were unimportant.

At autopsy on this animal there was no evidence of an acute or general infection, bacteriological examination revealing the presence of typhoid bacilli in only the sites of subcutaneous inoculation, the liver and bile. The most important tissue changes were those presented by the kidneys. They were strikingly altered in their naked eye appearance, the surface was roughened by elevations and depressions, but the capsules was not adherent and stripped off clean and easily. The color was not markedly altered.

Sections of these organs, hardened in alcohol, present features corresponding with the gross appearances. The areas of depression on the surface correspond to foci of proliferating fixed connective tissue elements. A considerable number of small granulation cells and larger fibroblasts occupy these areas, obscuring many of the tubules and compressing others. The glomeruli were likewise surrounded by such cells and in addition, the capsule of Bowman was at many points much

thickened. These areas, which were usually triangular in shape, with the bases at the capsule, were much contracted, the partially atrophied glomeruli being, by this means brought closer together. The thickening seen in the capsule of Bowman is due to the presence of an excess of fibrous tissue, poor in cellular elements. Adjoining the atrophic areas the tubules were seen to be dilated and the epithelium lining then was swollen and degenerated. In a few tubules hyaline casts were observed. In short, the condition was identical to that seen in the kidneys of human beings dead from chronic Bright's disease. (See photograph No. 1 and No. 2.)

The next animal, Rabbit V, was confined in the same chamber and kept under similar respiratory conditions. It lived 21 days, during which interval fluid cultures or suspensions of the *bacillus typhi abdominalis* were injected into its circulation by way of the ear vein on three occasions, 1.5 c.c. having been used for each injection. The animal died on the day following the last injection as did a control animal not exposed to the gases of decomposition.

During the 21 days that it was under observation its weight fell from 1080 to 995 grams and its temperature fluctuations were insignificant. At autopsy typhoid bacilli were recovered from the liver, and spleen; the blood, urine and kidneys being negative. As with pig A the conspicuous alterations in this animal were those found in the kidneys. They presented upon the cortical surface a remarkable mottled appearance, being thickly studded with round, pale, flat areas that looked to the naked eye like minute cysts, about the size of a pin point. A number of small red points were also seen. The capsule stripped off easily and clean. On section the cortical portion is red, the medullary pale. Sections of these organs, hardened in absolute alcohol, revealed conditions suggestive of the action of a very energetic poison. Unlike the chronic conditions found in pig A, there was here an acute process. Everywhere throughout the entire organ the intertubular capillaries were literally plugged with polynuclear leucocytes, causing them to stand out in bold contrast with the tubular elements, particularly the convoluted tubules. The epithelium of the convoluted tubules was granular and necrotic, the nuclei did not take up the staining and in a few of these tubules fibrin was demonstrated by the method of Weigert. It is interesting to note that this necrosis was especially seen in the epithelium of the convoluted tubules and did not extend to that of the collecting tubes, the epithelium of which showed but little deviation from the normal. At many points of contact this difference was conspicuous. The nuclei of both parenchymatous and interstitial elements frequently gave evidence of the action upon it of

a violent poison, by destructive changes of varying degree that were present at different points. The glomerular nuclei were conspicuously affected, being fragmented, distorted, pinched off into buds or drawn out into long thread-like processes and otherwise altered in shape. Many of the glomerular capillaries were hyaline and at some points there was an accumulation of granular matter within the capsule of Bowman. The entire process was one that suggested the activity of a violent toxic agent. (See photograph No. 3.)

Control animals inoculated in different ways with cultures of the typhoid bacillus showed at autopsy no appreciable alterations in the kidneys.

Two guinea pigs were then placed in the chamber and subjected to the gaseous products of decomposition alone; not being inoculated at all.

The experiment was begun April 11th, 1894 and continued with no interruption for 27 days, when, on the night of the last day the aspirator ceased to work and both animals were asphyxiated during the night.

At autopsy there was no macroscopic evidence of decomposition, nor did sections of the organs show such change. There was nothing abnormal about the kidneys of either animal to the naked eye.

They were placed in absolute alcohol and in Fleming's solution. In addition to the changes that will be described from the alcohol preparations those from Fleming's solutions showed the presence of a few minute black points, doubtless fat, in the epithelium of the convoluted tubules.

Further examination of sections from the kidneys of both animals showed a striking increase in the number of nuclei apparently between the tubules and surrounding the glomeruli in the cortex. The increase of nuclei was not equally present in all parts, nor was it present to the same degree in both animals.

In pig K, in which the change was less marked, the increase was apparently limited to the blood vessels. Large areas of the sections often showed no deviation from the normal, whereas along the distribution of one or two intertubular arteries the increase of nuclei was apparent. For the most part the cells presented the appearance of fibroblasts, were possessed of epitheloid nuclei and in no instance could fibres be made out between them. Sometimes the increase was especially about the glomeruli, resulting in an apparent thickening of the capsule, at other times it was more marked between the tubules.

In the case of pig J (the companion of the preceding animal), the changes resulting from cellular proliferation in the cortex are still more marked. (See photograph No. 4.) In the first place the areas of cellu-

lar proliferation are much more numerous and the distribution is somewhat different. Such localizations as have just been described in the companion animal (pig K) namely: the increase of cells along the cortical vessels, is found here also, but in addition there are small groups of cells just beneath the capsule, extending a short distance downwards into the substance of the kidney, and associated at these points with a slight indentation of the surface of the organ. In this animal, as little as in the other, could fibres be made out distinctly between the cells. The Fleming preparations showed, as did those of the other animal, a small amount of fat in the epithelium of the convoluted tubules. Beyond this it cannot be said that marked changes in the epithelium were demonstrable. A control guinea pig of the same lot, killed for purposes of comparison, showed in the kidney lesions that are similar to those described in the last two animals, although they are less marked than in the latter (pig J) and more marked than in the former (pig K). In fact they correspond so closely in many respects with the lesions seen in pig K that we feel further investigations upon new lots of animals to be necessary before definite conclusions can be drawn. If, however, one is justified in formulating an opinion from the results that have been obtained in these experiments, we think it evident that they suggest the existence in the concentrated emanations from decomposing matters of substances which when respired, have the power of producing alterations in the integral elements of the kidneys of animals, but we do not, as yet, consider this point demonstrated.

It is equally interesting that animals can live for so long a time in such an atmosphere and experience no greater pathological lesions than those to which allusion was made in guinea pigs J and K. If the results of experiments made upon animals in this way are of any value in demonstrating the positive or negative effect of air saturated with gaseous products of decomposition, it does not seem reasonable from these studies to suppose that the air of a sewer or cesspool, in the enormous dilutions that it exists at the time it reaches an individual in a house to which it has access, can be of much importance in either the direct production of diseased conditions or, in alone influencing the vital powers of resistance of the individual who inhales it.

NOTE.—Before closing I wish to acknowledge my indebtedness to Dr. Simon Flexner, Associate in Pathology, Johns Hopkins University, for his valuable assistance in the study of the pathological conditions found in the animals upon which the above experiments were performed.

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PHOTOGRAPHS.

- No. 1—Section through kidney of Guinea Pig A ; showing condition of chronic interstitial nephritis. $\times 125$.
No. 2—Section through another portion of same kidney. $\times 60$.
No. 3—Section through kidney of Rabbit V ; showing accumulation of polynuclear leucocytes in the intertubular capillaries and necrotic condition of tubular epithelium. $\times 125$.
No. 4—Section through kidney of Guinea Pig J ; showing accumulation of fibroblasts at points about the glomeruli ; and in some places apparent thickening of the capsule of Bowman. $\times 125$.

NOTE.—I am under many obligations to Doctor Walter Reed, Surgeon U. S. Army, Curator of the Army Medical Museum, Washington, D. C., for his kindness in having had these photographs made for me.

TABLE I.

Showing condition of the air over decomposing substances confined in closed vessels, after varying intervals. All maintained at the ordinary temperature of the laboratory.

Date.	Material.	Period of confinement in flask.	Oxygen, as vol. per cent.	Total gases absorbed by KOH. Vol. per cent.	Remarks.
Oct. 5, 1893.	Sewage.	12 days.	17.25	1.42	No distinct odor, neutral reaction.
Nov. 8, 1893.	"	35 "	13.39	4.12	" " " "
Feb. 12, 1894.	"	35 "	6.50	14.4	Consists of slime, etc., scraped from the bottom of sewers and mixed with fluid sewage.
April 23, 1894.	"	7 "	0.45	23.63	Consists of slime, etc., scraped from the bottom of sewers and mixed with fluid sewage.
Oct. 1, 1893.	Meat infusion.	8 days.	0.27	34.8	These infusions consisted of about 50 grams of finely chopped lean meat to a litre of water.
Nov. 4, 1893.	"	15 "	1.54	19.28	All samples, including the sewage samples, were kept in hermetically sealed flasks and were only opened for analysis. They were all maintained at the ordinary temperature of the laboratory.
April 23, 1894.	"	7 "	0.22	29.38	

TABLE II.

Analyses of air passing continuously over decomposing infusion of meat confined in flask.

Date.	Litres of air drawn through during preceding day	Rate of renewal of air in flask for preceding day.	Total CO ₂ expressed as milligrams.	Total H ₂ S as milligrams.	Relative CO ₂ as milligrams per litre.	Relative H ₂ S as milligrams per litre.	Temperature fluctuations in degrees C.		
Oct. '93									
23	58.3	19.4	82.2	35.8	1.4	0.103	23	{ Note preceding fall of temperature. Observe its effect on H ₂ S. Compare with chart.	
24	58.3	19.4	82.2		1.4		20		
25	69.6	23.2	156.0		2.24		20.5		
26	72.0	24.0	182.5		2.53		16		
27	90.0	30.0	376.2		4.18		21		
28	69.6	23.2	183.0	12.6	2.63	0.029			{ Note effect of fall in temp. upon H ₂ S. Compare with chart.
29	90.5	30.2	185.3		2.05				
30	90.5	30.2	185.3		2.05				
31	----	----	----		----				
Nov.									
1	186.5	62.2	326.8	12.6	1.75	0.029		{ Fall in temp. Note effect on H ₂ S. Compare with chart.	
2	193.9	64.6	423.4	16.2	2.18	0.084	26		
3	110.6	36.9	602.8	11.0	5.45	0.099	21		
4	148.8	49.6	491.2	6.1	3.30	0.041	17.5		
5	148.9	38.3	465.6	2.4	3.13	0.016	13		
6	66.5	22.2	176.7	69.0	2.66	1.038	24		
7	74.1	24.7	526.2	0.00	7.11	0.00	23		
8	4.26	1.42	279.1	1.1	65.5	0.258	20		
9	5.16	1.72	398.5	0.3	77.2	0.058	22		
10	4.08	1.36	312.8	0.00	76.7	0.00	21.5		
11	6.10	2.03	374.2	0.00	61.4	0.00	23	{ Flask sealed since 23d.	
12	4.30	1.43	263.0	8.7	61.2	2.023	10		
13	5.54	1.85	244.1	4.7	44.1	0.848	15.5		
14	5.41	1.80	251.5	0.4	46.5	0.074	24		
15	58.0	19.3	449.7	0.00	7.8	0.00	16		
16	53.6	17.9	145.6	0.1	2.72	0.002	8		
17	101.5	33.8	270.5	0.8	2.67	0.008	25		
18	87.	29.0	214.5	3.60	2.47	0.042	26		
19	147.	49.0	246.5	5.1	1.68	0.035	22		
20	73.8	24.6	152.7	0.00	2.07	0.00	24		
21	55.6	18.5	237.3	5.5	4.27	0.099	21	{ Flask sealed since Nov. 25. Note effect of reduction in temp. on H ₂ S.	
22	128.0	43.0	275.9	7.1	2.16	0.06	25		
23	129.4	43.1	236.2	13.2	1.83	0.102	26		
24									
25	$\alpha=33$	11	233.0	1.4	----	----	25		
	$\beta=9$	9	113.0	4.5	----	----	25		
Dec.									
2	α 9	3	425.	1.8	----	----	22		
	β 9	3	117.	5.0	----	----	15		

TABLE III.

Analyses of air passing continuously over sewage confined in flask.

Date.	Litres of air drawn through during preceding day.	Rate of renewal of air in flask for preceding day.	Total CO ₂ expressed as milligrams.	Total H ₂ S expressed as milligrams.	Relative CO ₂ as milligrams per litre.	Relative H ₂ S as milligrams per litre.	Temperature fluctuations in degrees C.	
Dec'93.								
11	91.6	30.5	108.	2.3	1.2	0.03	22	{ After analysis of 14th flask was gently agitated and dense pellicle that had formed on the surface of the sewage was ruptured. Note effect on H ₂ S. See chart.
12	84.8	28.3	92.4	2.2	1.1	0.03	24	
13	89.5	29.5	123.2	7.5	1.4	0.084	21	
14	82.0	27.3	94.7	0.00	1.2	0.00	20	
15	124.5	41.5	114.0	61.5	0.92	0.494	24	
16	110.5	36.8	103.7	12.9	0.94	0.120	26	
17	242.0	80.6	106.2	28.7	0.44	0.12	19	
18	167.9	55.9	57.6	12.9	0.34	0.08	18	
19	---	---	---	---	---	---	---	
20	177.7	59.2	99.3	3.1	0.56	0.017	21.5	
21	163.	54.3	58.2	0.00	0.36	0.00	22	{ Note effect of sudden fall in temp. in causing increase of H ₂ S. See chart.
22	---	---	---	---	---	---	---	
23	80.5	26.8	105.5	3.8	1.31	0.48	21	
24	129.9	43.3	83.4	16.3	0.642	0.13	23	
25	73.9	24.6	51.4	16.7	0.70	0.21	20	
26	145.0	48.3	135.5	72.5	0.94	0.50	12	
27	123.0	41.0	44.3	18.6	0.36	0.15	20	
28	245.3	81.8	---	---	---	---	20	
29	37.5	12.5	26.0	21.2	0.61	0.57	23	
30	170.8	56.9	53.0	71.5	0.31	0.42	18	

Sample 2, from another sewer.

Jan. '94								
10	86.5	28.8	354.9	1.3	4.10	0.015	21.5	{ Sudden fall in temp. Note effect on H ₂ S.
11	98.5	32.8	245.4	0.7	2.50	0.007	19	
12	107.3	35.8	209.7	0.00	2.00	0.00	15	
13	185.2	61.7	242.8	0.00	1.31	0.00	18	
14	120.	40.	84.4	0.6	0.70	0.005	19-14	
15	58.2	19.4	35.4	1.9	0.61	0.033	22	
16	65.	22.	77.5	43.3	1.21	0.67	24	
17	181.6	60.5	136.4	38.8	0.751	0.22	23	
18	181.2	60.4	81.1	59.0	0.48	0.33	22.5	
19	156.3	52.1	116.4	40.6	0.75	0.26	24	

TABLE IV.

Fractional distillations at 100° C., to determine the rate at which free ammonia and substitution products of ammonia pass off from solutions containing them.

Material tested.	Amount.	Amount of water or diluent employed.	Distillation with or without boiling stone.	Contents of each fraction of 50 c.c. of distillate.							Total NH_3 found.	Correction for NH_3 in water employed.	Correct'd total.
				1st	2d	3d	4th	5th	6th	7th	8th		
A	I	=1 mg. NH_3	With	0.900	0.175	0.033	0.010	0.002	0.000		1.120	0.0185	1.1015
	II			0.900	0.175	0.040	0.007	0.0025	0.000		1.1245		1.106
B	I	=5 mgs. NH_3	With	3.250	1.300	0.475	0.170	0.055	0.018	0.006	0.002	0.027	5.254
	II			3.250	1.200	0.5250	0.170	0.055	0.014	0.0055	0.000		5.1925
C	I	=1 mg. NH_3	With	0.750	0.250	0.090	0.038	0.0105	0.0025	0.000	0.000	0.0425	1.0985
	II			0.800	0.1900	0.090	0.038	0.0105	0.0025	0.000	0.000		1.0885
D	I	=5 mgs. NH_3	Without	3.250	1.200	0.3125	0.075	0.024	0.006	0.0015	0.000	0.0295	5.0395
	II			3.500	1.150	0.300	0.060	0.012	0.006	0.000	0.000		4.9985
E	I	=1 mg. NH_3	Without	0.830	0.220	0.065	0.019	0.0035	0.003			0.0095	1.131
	II			0.8300	0.1900	0.045	0.013	0.0025	0.000				1.071
F	I	=5 mgs. NH_3	Without	2.750	1.200	0.550	0.2438	0.0875	0.045	0.016	0.0045	0.0145	4.8823
	II			2.900	1.100	0.575	0.2375	0.0563	0.0365	0.011	0.0035		4.9053
G	I	=1 mg. NH_3	Without	0.600	0.325	0.125	0.045	0.014	0.005	0.0025	0.002	0.0155	1.103
	II			0.650	0.275	0.125	0.055	0.0225	0.012	0.0025	0.0015		1.128
H.	Trimethyl-amine.	5 c.c. of about 1 per cent. sol.	With	1.150	0.2875	0.085	0.017	0.003	0.000		1.5425		
	Methylamine.	5 c.c. of about 1 per cent. sol.	With	1.050	0.240	0.070	0.014	0.000	0.000		1.3740		
I	Methylamine.	20 c.c. of about 1 per cent. sol.	With	3.500	1.050	0.2375	0.0650	0.0140	0.0035		4.870		
	Trimethyl-amine.	20 c.c. of about 1 per cent. sol.	With	4.8260	1.300	0.2933	0.055	0.0095	0.000		6.5038		
	Mixture of Methylamine.	5 c.c. of 1 per cent. sol.											
	Trimethyl-amine and Ammonium chloride	5 c.c. of 1 per cent. sol. 2½ mgs. NH_3	With	4.400	1.050	0.2850	0.070	0.013	0.0045		5.8225		

TABLE V.

ESTIMATION OF FREE AND ALBUMINOID AMMONIA IN AIR FROM DIFFERENT SOURCES. RESULTS EXPRESSED AS FRACTIONS OF A MILLIGRAM.

(1) Date.	(2) Source of air analyzed.	(3) Amount of air analyzed, in litres.	(4) Time consumed (minutes) in aspirating.	(5) Total ammonia found.		(6) Ammonia in water used. To be deducted.		(7) Amount of ammonia in air analyzed.		Remarks.
				Free.	Alb.	Free.	Alb.	Free.	Alb.	
Dec. 23, 1893	Outside air.	129.9	24	0.018	0.0025	0.0145	0.0025	0.0035	0.000	The air obtained for these analyses came from just outside a window of the laboratory, on the second floor, about 35 or 40 feet from the ground.
Dec. 23, 1893	"	35.2	25.5	0.0155	0.0025	0.0145	0.0025	0.001	0.000	
Dec. 24, 1893	"	230.8	49	0.0145	0.0025	0.0145	0.0025	0.000	0.000	
Dec. 24, 1893	"	47.6	50.5	0.0145	0.0025	0.0145	0.0025	0.000	0.000	
Dec. 26, 1893	"	355.4	48	0.0095	0.0018	0.006	0.0018	0.0035	0.000	
Dec. 26, 1893	"	108	46.5	0.0075	0.0025	0.005	0.0025	0.0025	0.000	
Dec. 28, 1893	"	56.1	20.5	0.0075	0.0025	0.005	0.0025	0.0025	0.000	
Dec. 28, 1893	"	182.2	25.5	0.0073	0.0025	0.005	0.0025	0.0023	0.000	
Dec. 13, 1893	Air of laboratory	31.4	2.5	0.009	0.009	0.009	0.009	0.000	0.000	The air used for these analyses was from my own room in the laboratory.
Dec. 21, 1893	"	105.3	22	0.0155	0.003	0.0145	0.003	0.001	0.000	
Dec. 22, 1893	"	76.3	4.5	0.0175	0.003	0.0145	0.003	0.003	0.000	
Dec. 29, 1893	"	169.5	22.5	0.008	0.0025	0.008	0.0025	0.000	0.000	
Dec. 29, 1893	"	207.1	21.5	0.009	0.002	0.0085	0.002	0.0005	0.000	
Dec. 30, 1893	Air from sewage.	36.9	5	0.036	0.0025	0.008	0.0025	0.028	0.000	In these experiments the air was aspirated over sewage contained in a flask. About four litres of sewage was placed in an eight litre flask for the experiments.
Dec. 30, 1893	"	141.2	5	0.008	0.004	0.0085	0.002	0.000	0.002	
Jan. 3, 1894	"	36.1	4	0.072	0.0065	0.008	0.0025	0.064	0.004	
Jan. 3, 1894	"	108	4.5	0.010	0.004	0.008	0.0025	0.002	0.0015	
Jan. 13, 1894	"	254.7	42.5	0.042	0.0023	0.0165	0.0023	0.0255	0.000	

TABLE V—Continued.

ESTIMATION OF FREE AND ALBUMINOID AMMONIA IN AIR FROM DIFFERENT SOURCES. RESULTS EXPRESSED AS FRACTIONS OF A MILLIGRAM.

(1) Date.	(2) Source of air analyzed.	(3) Amount of air taken, in litres.	(4) Time consumed (in hours) in aspirating.	(5) Total ammonia found.		(6) Ammonia in water used. To be deducted.		(7) Amount of ammonia in air analyzed.		Remarks.
				Free.	Alb.	Free.	Alb.	Free.	Alb.	
Jan. 8, 1894	Air from sewage.	61.9	19	0.0525	0.0025	0.008	0.0025	0.0445	0.000	In these experiments the air bubbled through the sewage before passing to the absorber.
Jan. 4, 1894	"	320	51	0.4265	0.0045	0.0095	0.0025	0.4170	0.002	
Jan. 4, 1894	"	866	48	0.590	0.0075	0.0095	0.0025	0.5805	0.005	
Jan. 7, 1894	"	284.5	22	0.1755	0.0039	0.0143	0.0038	0.1612	0.0001	
Jan. 7, 1894	"	387.7	23	0.2740	0.0038	0.0143	0.0038	0.2597	0.000	
Jan. 9, 1894	Air from decompos. meat infusi'n	337	24	I 4.9815	0.0115	0.009	0.0038	4.9725	0.007	=absorber I } In these experiments the air was
Jan. 11, 1894	Air from decompos. meat infusi'n	192.4	20	II 2.179	0.014	0.009	0.0038	2.170	0.0102	" II } drawn through vessels containing decomposing infusions of meat. It did not bubble through
Jan. 12, 1894	Air from decompos. meat infusi'n	203.8	24	I 1.205	0.0025	0.012	0.0012	1.193	0.0013	" II }
				II 1.150	0.0025	0.012	0.0012	1.138	0.0013	" II }
				I 2.521	0.003	0.0165	0.0023	2.5045	0.0007	=absorber I } the fluid but passed over the
				II 1.8815	0.0045	0.0165	0.0023	1.865	0.0022	" II } surface. For, each determination a group of more than one
Jan. 15, 1894	Air from decompos. meat infusi'n	178.3	24	I 4.821	0.0115	0.0155	0.0023	4.8055	0.0092	" I } absorber was employed.
				II 1.806	0.004	0.0155	0.0023	1.7905	0.0017	" II }
				III 0.030	0.005	0.0155	0.0023	0.0145	0.0027	" III }
Jan. 8, 1894	Air bubbling through decomposing meat infusion, 12 ds. old	143.2	21	I 1.549	0.0055	0.009	0.0038	1.540	0.0017	=absorber I }
Jan. 16, 1894	Air drawn over sewage.	124.5	20	II 0.1715	0.004	0.009	0.0038	0.1625	0.0002	" II }
				I 0.040	0.0015	{ 0.0165 0.0025 }		0.0235	0.000	=absorber I }
				II 0.0175	0.0013			0.001	0.000	" II }
				III 0.0145	0.001			0.000	0.000	" III }
Feb. 17, 1894	Air bubbling through sewage.	200	21	I 0.0620	0.000	{ 0.0095 0.000 }		0.0525	0.000	absorber I }
				II 0.0155	0.000			0.006	0.000	" II }
				I 0.323	0.002			0.3135	0.002	absorber I }
Feb. 18, 1894	Air bubbling through sewage.	259	25.5	II 0.012	0.000	{ 0.0095 0.000 }		0.0025	0.000	" II }

TABLE VI.
Transportation of bacteria by air bubbling through decomposing solutions.

	Length of tubes in centimeters.		Distance from culture to sterilized medium in cm.	Calibre of tubes in millimeters.		Litres of air aspirated.	Time consumed in aspirating.	Velocities of air current in tubes, as cm. per min.		Remarks. Results.
	Main.	Connections.		Main.	Connections.			Main.	Connections.	
1	90	40	130	15	5	7	45 minutes	88	798	Bacteria carried over, 6 colonies.
2	60	40	100	15	5	7	45 minutes	88	793	" " " 5 "
3	140	90	230	15	5	7	90 minutes	44	397	" " " "
4	200	40	240	15	5	7	90 minutes	44	397	" " " "
5	90	40	130	15	5	7	120 minutes	33	297	" " " "
6	60	40	100	15	5	7	240 minutes	16.5	149	" " " "
7	140	90	230	15	5	5½	360 minutes	8.6	78	No bacteria carried over.
8	90	40	130	15	5	7	480 minutes	8.3	74	" " " "
9	90	40	130	15	5	4	360 minutes	8.0	57	" " " "
10	60	40	100	15	5	3½	420 minutes	4.8	42.5	" " " "
11	----	----	448	15	--	7	30 minutes	134	----	" " " "
12	----	----	448	15	--	7	15 minutes	268	----	" " " "
13	----	----	448	15	--	7	15 minutes	268	----	" " " "
14	----	----	448	15	--	7	15 minutes	268	----	Bacteria carried over.
15	----	----	448	15	--	7	15 minutes	268	----	" " " "

CHART I

Showing tension of air over sewage as compared with that of air over decomposing infusion of meat. All kept under same conditions.

- Tension of air over decomposing meat infusion, expressed in millimeters of mercury. Infusion confined in hermetically sealed flask. Experiment of Sept. 22nd, 1893.
- " " " " Sewage from South St. sewer " " " " " " " " " " Experiment of Sept. 26th, 1893.
- Fluctuations of temperature in degrees Centigrade.
- o-o- Tension of air over mixture of fluid sewage and slime scraped from sides and bottom of sewer. Mixture confined in hermetically sealed flask.
- o-o-o- Temperature curve for preceding experiment.

September, 1893.

October.

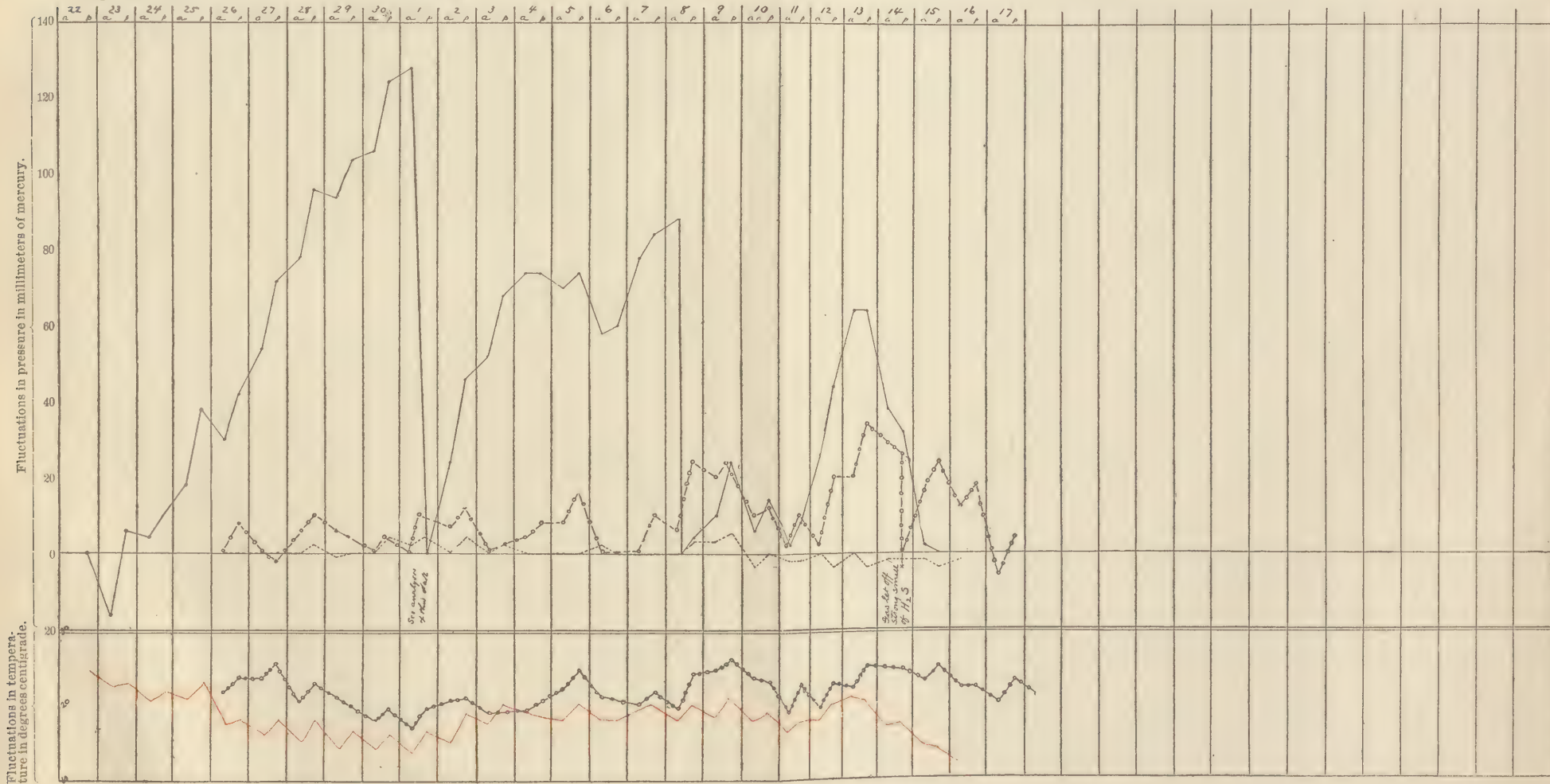


CHART II

Showing absolute and relative amounts of Carbonic Acid and Sulphuretted Hydrogen taken up by air in passing over an actively decomposing infusion of meat.

KEY :

- Rate of ventilation expressed as the number of times the entire volume of air in the flask (3 liters) was renewed during the preceding 24 hours.
- Total amount of CO_2 given off during preceding 24 hours ; expressed in milligrams.
- Relative amount of CO_2 expressed as milligrams per liter ; one milligram per liter at 20°C . being equivalent to about 10.7 parts per 10,000 of air (per volume).
- Total amount of H_2S given off during preceding 24 hours ; expressed in milligrams.
- Relative amount of H_2S expressed as milligrams per liter ; one milligram per liter at 20°C . being equivalent to about 7.34 parts per 10,000 of air (per volume).
- Fluctuations of temperature, expressed in degrees Centigrade.

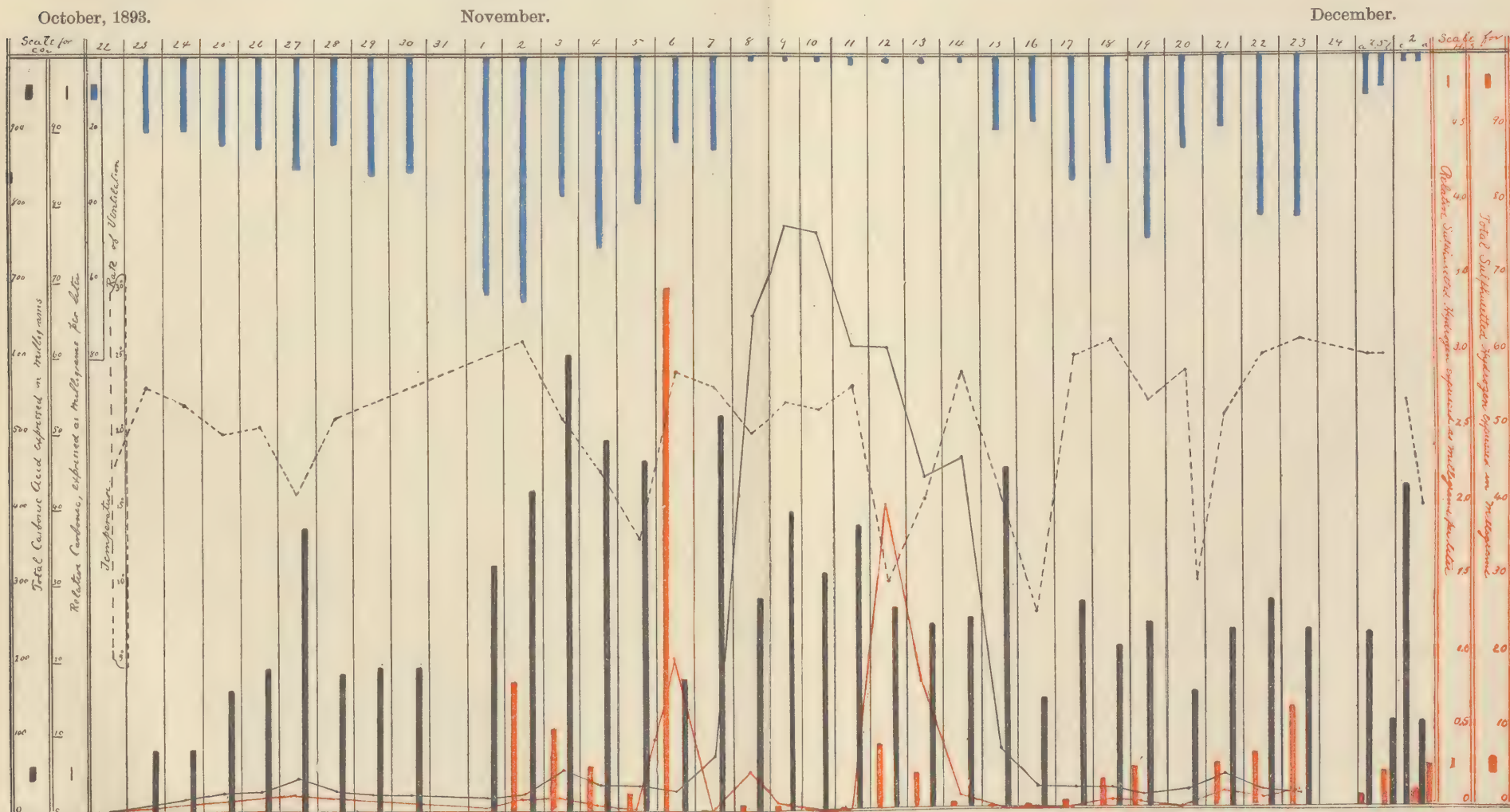
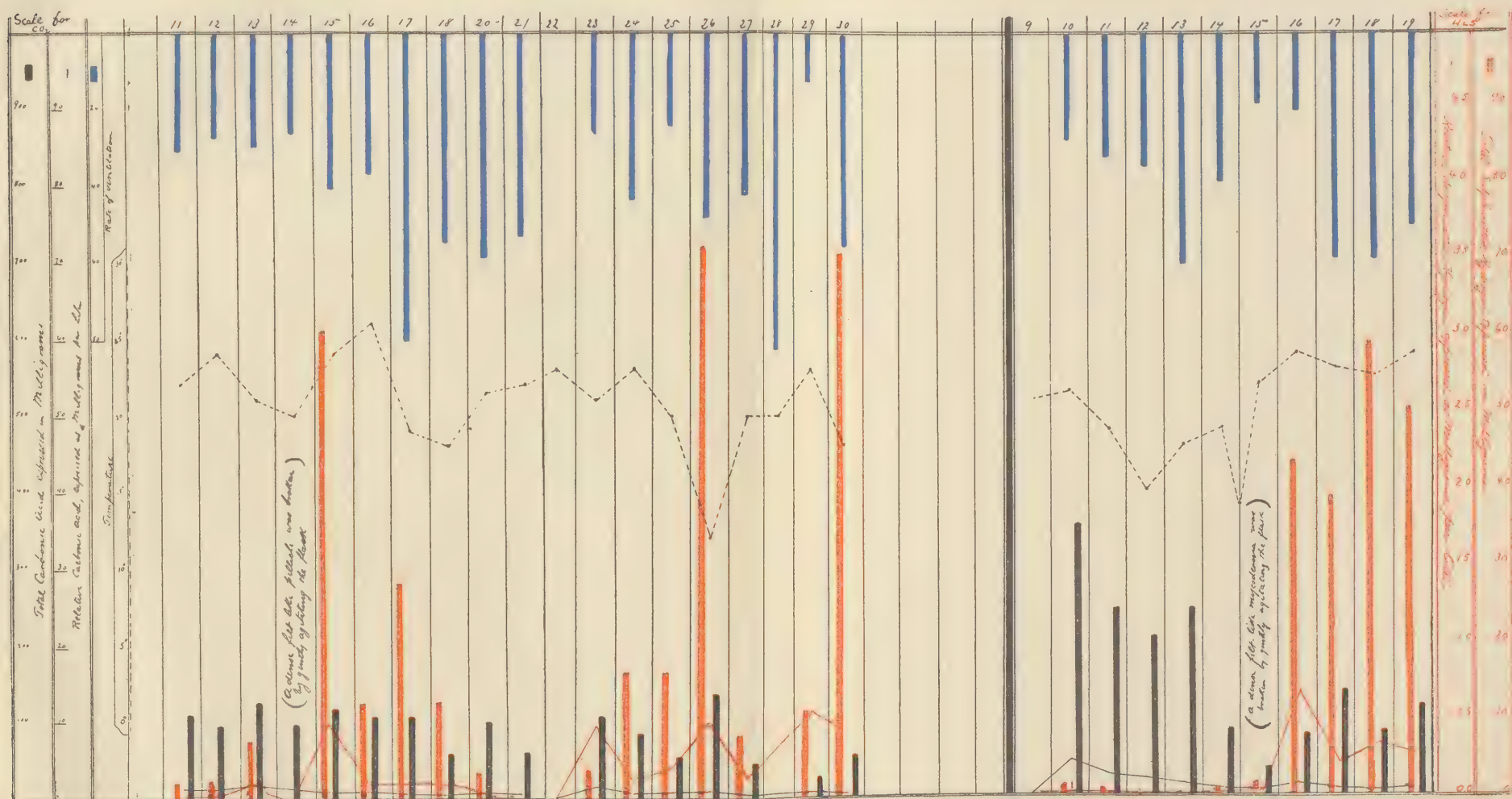
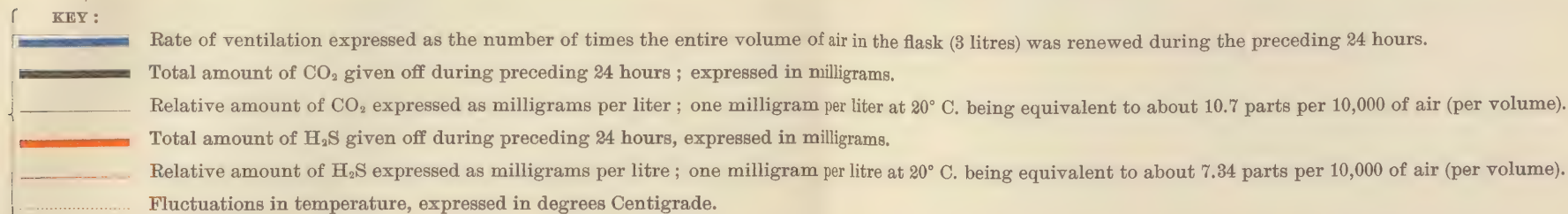
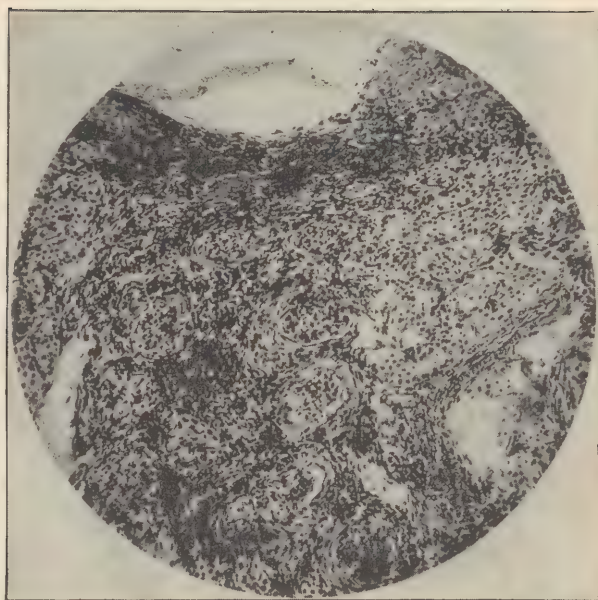


CHART III

Showing absolute and relative amounts of carbonic acid and sulphuretted hydrogen taken up by air in passing over sewage confined in flasks at the ordinary temperature of the room.

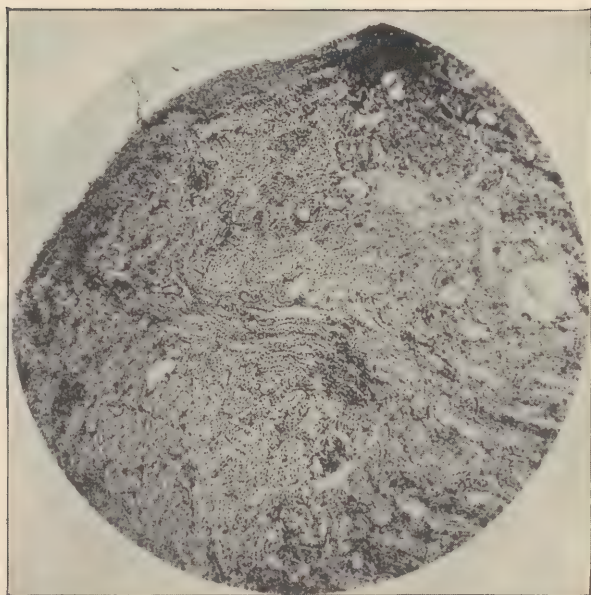






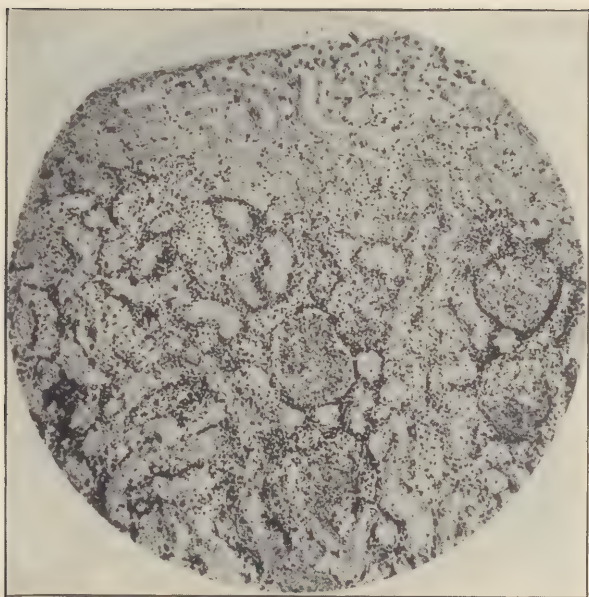
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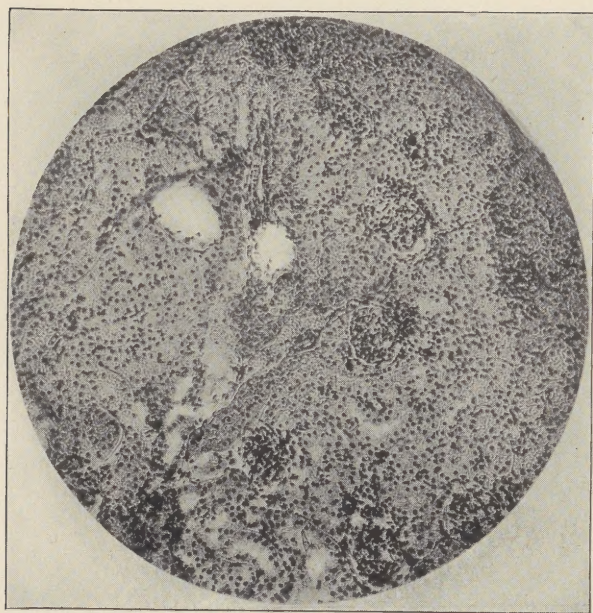
No. 2. $\times 60$.





No. 3. $\times 125$.





No. 4. $\times 125$.

